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Entrapping Method Surface Modification for Preparation of Membrane Adsorber

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Abstract

Ion-exchange chromatography in packing bed is one of techniques widely used in the purification of protein. However, this technique has some limitations such as intraparticle diffusion of solute transport, high pressure drop; radial and axial dispersion limitation and chanelling. These factors make scale up of packed bed chromatographic processes difficult. Recently, membrane adsorber has been introduced as an alternative to overcome these problems. In this work, surface modification with UV irradiation using photoinitiator entrapping method was used to produce membrane adsorber. This is based on the simplicity and the economic value of such method [1,2]. Polypropylene microporous membrane was used as substrate, benzophenone was used as photoititiator in different range of concentration (0.01-1 wt %) and Acrylic acid (AA); Acrylamide (AAm) and N,N'-methylenebisacrylamide (MBAA) were used as monomer in different mixture of composition. Degree of grafting, permeability, ATR-FTIR, and protein reversible binding were used as methods of evaluation. Membrane adsorber produced with photoinitiator 0.1wt% yielded optimum degree of grafting and combination of Acrylic acid and low amount N,N'-methylenebisacrylamide showed highest protein reversible binding. The overall results showed variations of photoinitiator concentration and monomer composition had significant influence on membrane adsorber performance.

Keywords: Membrane absorber, Surface modification, UV irradiation, Entrapping method

1.0 Introduction

Macroporous membranes as membrane adsorber had been proposed more than a decade ago in order to overcome the limitations of particle beds [1, 2]. The transport of solutes through the membrane pores can take place by convection, the pressure drops for high flow rates are much lower, and the scale up is rather easy. In the meantime, first commercial membrane adsorbers are on the market. However, the interplay of membrane pore size and distribution, affinity binding and flow rates is still not understood in all details, and hence the potential of porous affinity membrane adsorbers can not be fully exploited yet.

Hydrophilic membranes have good characteristic of low non-specific adsorption of proteins but have poor thermal stability and are susceptible to chemical agents. In contrast, hydrophobic membranes have good thermal stability and chemical resistance but high non-specific protein adsorption. Therefore, a modification of hydrophobic polymer membranes that introduces hydrophilic segments on the surface is an ideal method for combining the advantages of hydrophilic and hydrophobic membranes [1].

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Surface modification has become a key technology to improve the separation performance of already established membranes as well as to produce novel separation membranes. Photo-initiated graft copolymerization is a versatile approach to create chemically well-defined thin grafted polymer layers [3], e.g. on the entire internal surface of microfiltration membranes without damaging their matrix pore structure [4]. Such thin grafted polymer layers have impact onto the reduction of protein adsorption (fouling) [5]. Furthermore they can be used for the covalent immobilization of biomolecules [6]. One already established application of the latter approach is the affinity separation of proteins and other biomolecules by using porous affinity membrane adsorbers [6].

The aim of this work is to investigate systematically varied linear or crosslinked grafted functional polymer layers on the same porous polypropylene (PP) membrane by using the same functionalization method with optimum photoinitiator concentration. A new surface-selective photo-grafting method was used which is based on "entrapping" the photoinitiator in the surface layer of the PP membrane [7]. No initiator was added in the solution to avoid side reactions. In the present study, PP membranes with a cut-off pore size of ~0.4 µm were functionalized using acrylic acid, acrylamide and N,N'-methylene bisacrylamide. Degree of grafting, water permeability as a function of pH and protein adsorption under membrane chromatography conditions were measured, and it was found that the architecture of grafted cation exchange polymer layers has indeed a great influence on the performance of the functionalized membranes

2.0 Materials & Method

2.1 Materials

Polypropylene (PP) membranes (Accurel PP 2EHF, cut-off pore size ~0.4 µm, membrane thickness ~150 µm) were purchased from Membrana GmbH, Wuppertal. Acrylic Acid (AAc), benzophenone (BP) and lysozyme (Lys) were obtained from Fluka. Acrylamide (AAm) and N,N'-methylene bisacrylamide (MBAA) were purchased from Aldrich. The HEPES buffer was from Sigma-Aldrich. Heptane, methanol and NaCl were from Applichem. Sodium hydroxide and Hydrochloric acid were from Waldeck.

2.2 UV photoinitiated grafting

A membrane sample with a diameter of 25 mm was presoaked in 2 ml solution of BP (0.01, 0.1 and 1wt.%) in heptane for 1 hour. Then, it was taken out and dried in air for 10 min.. Thereafter, it was wetted in methanol for 5 min., and then wiped with a filter paper before it was put in the monomer solution for 30 min.. In the next step, the membrane was irradiated by using high intensity UV (UV-A Print, Hoenle, Gräfelfing, Germany) and a glass filter (>300 nm) for 15min. Thereafter, it was washed intensively with water and methanol before it was dried in an oven for 1 day. Then, the membrane weight was measured, and the degree of functionalization (DG) was calculated using the weight of the original membrane sample and the specific weight (normalized to the outer membrane surface).

2.2.1 For variation in photoinitiator concentration, 4 different photoinitiator concentrations were used (0.01, 0.1, 0.5 and 1 wt %). Acrylic acid was used as monomer with 1.5 g/L concentration

2.2.2 For variation in monomer mixture (functional, diluent, cross linker), optimum photoinitiator concentration was used (Table 1).

Table 1 Variation of monomer concentration and composition.

Type of Membrane	Monomer concentration in water			
	C Total (g/L)	AAc (g/L)	AAm (g/L)	MBAA(g/L)
PP-g-PAAc	15	15	-	-
PP-g-PAAc-AAm	20	10	10	-
PP-g-PAAc-LMBAA	15.75	15		0.75
PP-g-PAAc-HMBAA	13.75	12.5		1.25

2.3 Membrane permeability

An Amicon cell 8010 (Millipore) was used for permeability measurements with water adjusted to pH 2 or pH 10, by adding HCl or NaOH solutions, respectively. Each permeability value was obtained from an average of 5 data which was taken by collecting the filtrate for 30 sec. and determining its amount gravimetrically.

2.4 Membrane chromatography

The liquid chromatograph ÄKTApurifier (Amersham Pharmacia Biotech) was used. 3 membrane samples with a diameter of 12 mm were used as a stack in a CIM® module (BIA Separations, Ljubljana, Slovenia). Buffer A (10 mM HEPES, pH 7.0) was used for membrane equilibration, protein binding and subsequent washing, while buffer B (10 mM HEPES, pH 7.0 + 1 M NaCl) was used for elution. Detection wavelength was 280 nm. The gradient program was as follows: 0-3 min.: flow 1.0 mL/min Buffer A; 3-4 min.: flow 0.5 mL/min Buffer A; 4 min.: sample injection; 4-12 min.: flow 0.5 mL/min Buffer A; 12-16 min.: flow 0.5 mL/min linear gradient to buffer B; 16-19 min.: flow 1.0 mL/min buffer A.; 19 min.: end. A blank gradient was run as the first step, and then two injections of 1 ml solution of Lys (5 mg/mL in buffer A) followed. Calibrations were done by injection of Lys solutions in Buffer A with different concentrations using the CIM® module without membrane stack

3.0 Results and Discussion

3.1 Determination of optimum photoinitiator concentration

Variations of photoinitiator concentration with one type of monomer composition were done in order to investigate the photoinitiator concentration for achieving optimum degree of grafting (Fig. 1).

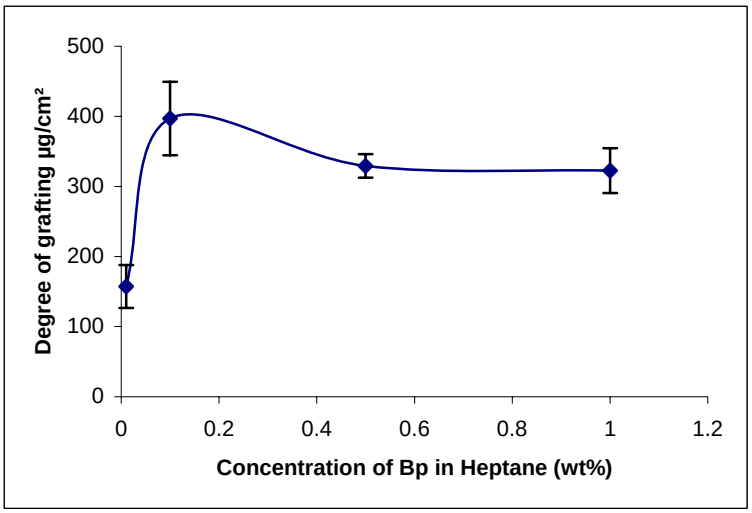

Figure 1 Degree of functionalization for PP membranes grafted with different photo initiator concentrations.

The variation of photoinitiator concentration showed a modest reproducibility (variation of the degree of grafting (DG) values around 20%). DG increased from 160 µg/cm² (at low photoinitiator concentration 0.01 wt%) to 400 µg/cm² (at photoinitiator concentration 0.1 wt%) and then slightly decreased to reach a plateau after that concentration.

Heptane has the ability to swell PP membrane moderately between ~ 7.6 wt% and 12 wt% at room temperature [8, 9]. This swelling behaviour was manipulated to entrap BP in the PP pores [10]. BP is photoinitiator and has a function to excite and facilitate formation of free radical on PP surface with the present of UV light. The amount of photoinitiator present in the pores is important because it will influence the photo grafting reaction. From the result above, 0.1 wt% photoinitiator concentration is found to produce optimum degree of grafting.

3.2 Degree of functionalization for membranes grafted with different polymer layers

Variations of the cation exchanger group (carboxyl) amounts and the grafted layer crosslinking were attempted by varied monomer composition used for photo-grafting. Taking into account the different monomer reactivities, the total monomer concentration was adjusted in order to obtain a similar degree of grafting, which should then allow investigating the influence of different grafted composition and architecture (Table 2).

The photo-grafting method showed a modest reproducibility, because the variation of the DG values had been always less than 20%. Among the four grafted membrane types, PP-g-AAc-AAm had the highest DG value while PP-g-PAAc and PP-g-PAAc-HMBAA had the lowest DG values. Overall, a rather uniform degree of functionalization had been obtained, with an average value of ~380 µg/cm².

Table 2: Degree of functionalization for PP membranes grafted with different polymer layers [11]

	Monomer concentration in water			Degree of functionalization DG (µg/cm²)	Var. coeff (%)	Number of samples
	AAc (g/L)	AAm (g/L)	MBAA(g/L)			
PP-g-PAAc	15	-	-	360±50	13.9	23
PP-g-PAAc-AAm	10	10	-	410±40	10.1	19
PP-g-PAAc-LMBAA	15	-	0.75	390±60	16	19
PP-g-PAAc-HMBAA	12.5	-	1.25	360±40	12	19

3.3 Membrane permeability and its response to pH change

The analysis of membrane permeability can yield information about the blocking of pores by the grafted polymer layers. Furthermore, due to the reversible deprotonation of carboxyl groups above the pK value (pH ~5), significant changes of the effective layer thickness can also be deduced from these data [4,7].

All grafted membranes had a high water permeability during filtration at pH 2 (Fig. 2), and the data were similar to the unmodified PP membrane (11,000 L/hm²bar). The PP-g-PAAc-HMBAA membranes had the highest permeability (13,900 L/hm²bar) while the PP-g-PAAc-AAm membranes showed the lowest values (12,300 L/hm²bar). Hence, all functionalized membranes seemed to have only slightly different effective pore size. The improved water permeability could be explained by the much higher hydrophilicity as compared with unmodified PP.

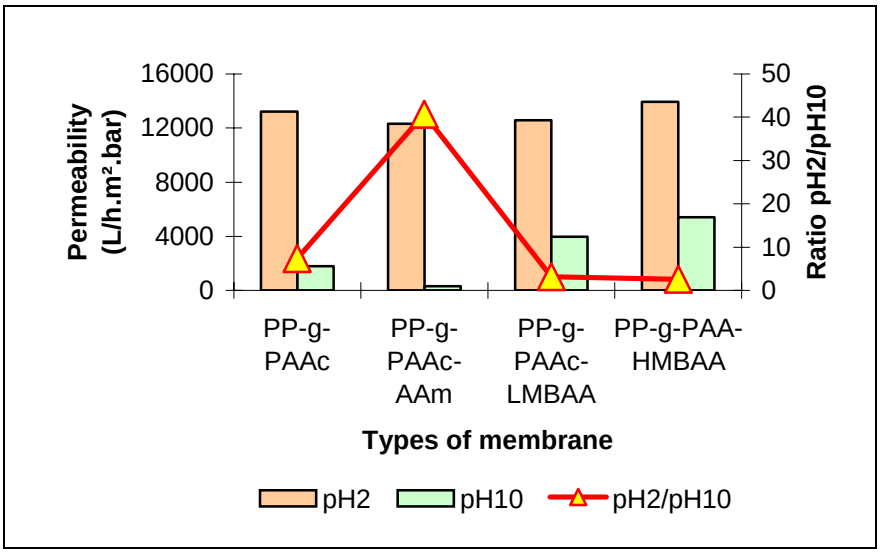

Figure 2 Water permeabilities and the permeability ratio pH 2 vs. pH 10 for membranes with different grafted surface architecture [11].

The permeability for all grafted membranes reduced significantly during filtration at pH 10. Interestingly, PP-g-PAAc-HMBAA still exhibited the highest permeability with 5,400 L/h.m².bar while the PP-g-PAAc-AAm membranes showed by far the lowest water permeability with 300 L/h.m²bar.

The reversible deprotonation of carboxylic groups in grafted polyacrylic acid segments leads to an ionic repulsion and an osmotic pressure, both forcing the polymer brush to stretch. This phenomenon leads to a decrease of effective membrane pore size, as a result a lower permeability is observed. For the membranes grafted with acrylamide copolymers of AAc, two additional effects should play a role, for PP-g-PAAc-AAm a "dilution" of carboxyl groups, and for the PP-g-PAAc-MBAA membranes the crosslinking of the grafted chains.

The somewhat surprising behaviour of the PP-g-PAAc-AAm membranes can be explained based on a higher (and pH-independent) swelling of the grafted PAAm segments as compared with the PAAc segments. Therefore, at high pH, the overall stretching of the copolymer brush is even larger than for PAAc homopolymer. At low pH, the formation of hydrogen bonds between PAAc and PAAm segments, present in about equal amounts, reduces the swelling below the degree what would have been expected by the contribution of the PAAm segments.

The most interesting finding was that the water permeabilities of the membranes prepared with the bisacrylamide crosslinker were significantly higher, especially at pH 10. Similar to what had been observed with other systems [12], the polymer network can limit the swelling / stretching of the polymer brush layer in the membrane pores, and the permeabilities increases. This effect can be tailored by the content of crosslinker in the monomer mixture.

3.4 Protein binding under membrane chromatography conditions

Reversible protein binding was evaluated using lysozyme, having a molecular weight of 13.9 kDa and an isoelectric point of about 11.9. Hence, at neutral pH, protein binding can oocur via cation-exchange with carboxyl groups in the grafted layer on the membrane pores, and elution should be achieved by high salt concentration (Table 3)

Table 3 Reversible protein binding of membranes with different grafted surface architecture (injected lysozyme amount 5.0 mg) [11].

Photo-grafted membrane	No. of injection	Lysozyme bound (mg)	Lysozyme eluted (mg)	Recovery (%)
PP-g-PAAc	1st	3.80	2.91	76
	2nd	3.79	3.03	80
PP-g-PAAc-AAm	1st	3.74	2.78	74
	2nd	3.65	2.89	79
PP-g-PAAc-LMBAA	1st	3.88	3.62	93
	2nd	3.78	3.83	101
PP-g-PAAc-HMBAA	1st	3.26	2.99	92
	2nd	3.22	3.16	98

Rather high amounts of Lys binding were observed for all membranes, corresponding to about 30 mg/ml bed volume. This is in the same range as reported for other membrane adsorbers [1,2], and it can only be explained by a three-dimensional "packing" of Lys in the grafted polymer layers. However, not in all cases, this amount could be quantitatively recovered in the elution peak.

Focusing on eluted Lys amounts, the membranes with crosslinked grafted layer had higher values, and the AAc-AAm copolymer layers had values similar to the AAc homopolymer. Also, the recovery was significantly higher for the crosslinked AAc-MBAA copolymer membranes; in the second runs ~100% of the bound Lys could be recovered. Overall, an optimum of protein separation performance (highest binding and recovery) had been observed for the PP-g-PAAc-LMBAA membranes, i.e. the grafted layers with a low crosslinker content.

It should be noted, that under the evaluation conditions for protein binding, the membranes were in their stretched grafted layer conformation as deduced from the permeability measurements. Hence, as compared with the homopolymer brush membranes (PP-g-PAAc), the lower carboxyl content in the PP-g-PAAc-AAm membranes could be compensated by a higher degree of swelling allowing higher uptake; however, the release under elution conditions was not efficient enough. At an optimum crosslinker content (similar to PP-g-PAAc-LMBAA), the cation exchange polymer brush layers were more compact than the uncrosslinked layers with PAAc segments in linear chains, but still did not impose major accessibility limitations for the protein as could be observed for too high crosslinking degree (PP-g-PAAc-HMBAA).

4.0 Conclusion

Optimum degree of grafting was obtained using 0.1 wt% photo initiator concentration. Photo-initiated surface functionalization of porous polypropylene membranes using a mixture of an acrylic acid, acrylamide and crosslinker yielded formation of polymer network layers, while using a mixture of monofunctional acrylic acid and acrylamide linear random copolymer brushes were obtained. Chemical crosslinking reduced the molecular mobility of the grafted brush layers and limited the swelling effects. As a consequence, the overall membrane performance was increased as compared with the linear structures. Therefore, at the same degree of functionalization and very similar composition, the surface layer architecture is the main factor to tailor membrane characteristics. Future work will focus on hydrodynamic model and adsorption behaviour of the novel membrane adsorbers.

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